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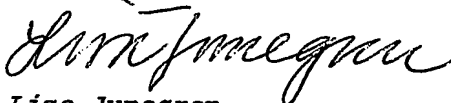
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TETRACYCLIC IMMUNOMODULATORY COMPOUNDS

Huvudtext

The present invention relates to novel heterocyclic compounds, to methods for their preparation, to compositions containing them, and to methods and use for clinical treatment of medical conditions which may benefit from immunomodulation, including rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis. More particularly the present invention relates to novel heterocyclic compounds, which are CD80 antagonists capable of inhibiting the interactions between CD80 and CD28.

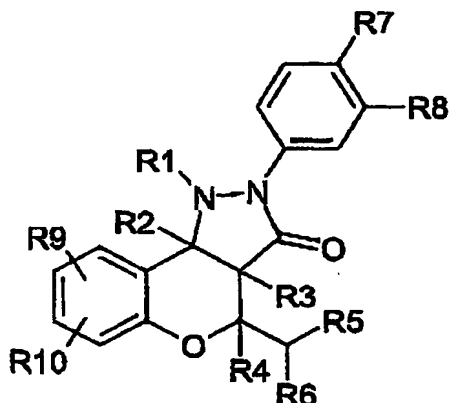
Background of the invention

The immune system possesses the ability to control the homeostasis between the activation and inactivation of lymphocytes through various regulatory mechanisms during and after an immune response. Among these are mechanisms that specifically inhibit and/or turn off an immune response. Thus, when an antigen is presented by MHC molecules to the T-cell receptor, the T-cells become properly activated only in the presence of additional co-stimulatory signals. In the absence of accessory signals there is no lymphocyte activation and either a state of functional inactivation termed anergy or tolerance is induced, or the T-cell is specifically deleted by apoptosis.

One such co-stimulatory signal involves interaction of CD80 on specialised antigen-presenting cells with CD28 on T-cells, which has been demonstrated to be essential for full T-cell activation. (Lenschow et al. (1996) Annu. Rev. Immunol., 14, 233-258)

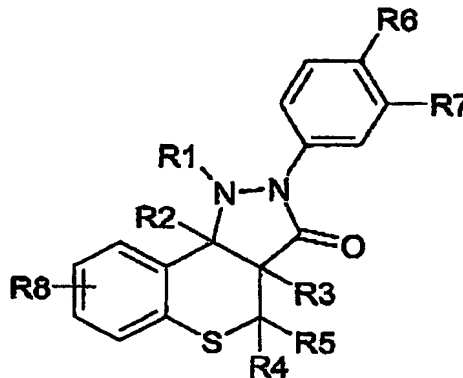
A paper by Erbe et al, in J. Biol. Chem. Vol. 277, No. 9, pp 7363-7368, describes three small molecule ligands which bind to CD80, and inhibit binding of CD80 to CD28 and CTLA4. Two of the disclosed ligands are fused pyrazolones of structures A and B:

3



D

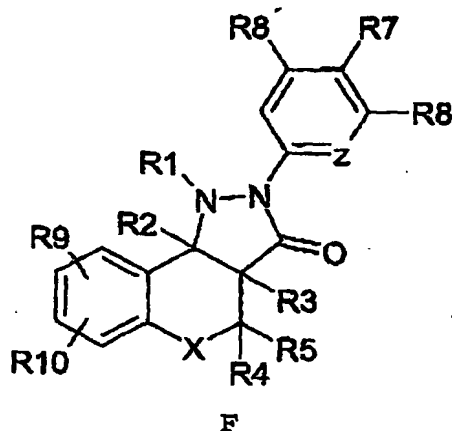
Similarly EP 0354694A1 (Boots) discloses immunomodulatory compounds of general structure E but here are not included structures wherein R6 and/or R7 are COOH or contain a COOH group.



E

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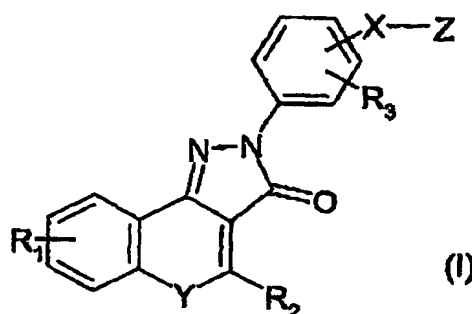
Also, WO9111448 (Boots) discloses immunomodulatory compounds of general structure F but here are not included structures wherein R7 and/or R8 and R8' are COOH or contain a COOH group.



F

Description of the invention

According to the present invention there is provided
a compound of formula (I) or a pharmaceutically or vete-
rinarly acceptable salt thereof:



(I)

wherein

Z represents a carboxylic acid group (-COOH) or an ester thereof;

R₁ and R₃ independently represent H; F; Cl; Br; -NO₂; -CN; C₁-C₆ alkyl optionally substituted by F or Cl; or C₁-C₆ alkoxy optionally substituted by F;

R₂ represents optionally substituted C₃-C₇ cycloalkyl or optionally substituted phenyl;

Y represents -O-, -S-, N-oxide, or -N(R₅)- wherein R₅ represents H or C₁-C₆ alkyl;

X represents a bond or a group selected from; a di-valent C₁-C₆ alkylene radical, NHC(O) C₁₋₅ alkyl, NHC(O) CH₂-O-CH₂ or C(O) -NH- (amino acid residue);

Compounds of general formula (I) are CD80 antago-
nists. They inhibit the interaction between CD80 and CD28
and thus the activation of T cells, thereby modulating
the immune response.

5 Accordingly the invention also includes:

(i) a compound of formula (I) or a pharmaceutically
or veterinarily acceptable salt thereof for use in the
treatment of conditions which benefit from immunomodu-
lation.

10 (ii) the use of a compound of formula (I) or a phar-
maceutically or veterinarily acceptable salt thereof in
the manufacture of a medicament for the treatment of con-
ditions which benefit from immunomodulation,.

15 (iii) a method of immunomodulation in mammals, in-
cluding humans, comprising administration to a mammal in
need of such treatment an immunomodulatory effective dose
of a compound of formula (I) or a pharmaceutically or ve-
terinarily acceptable salt thereof.

20 (iv) a pharmaceutical or veterinary composition com-
prising a compound of formula (I) or a pharmaceutically
or veterinarily acceptable salt thereof together with a
pharmaceutically or veterinarily acceptable excipient or
carrier.

25 Conditions which benefit from immunomodulation in-
clude:

- Acute disseminated encephalomyelitis
- Adrenal insufficiency
- Allergic angiitis and granulomatosis
- Amyloidosis
- 30 Ankylosing spondylitis
- Asthma
- Autoimmune Addison's disease
- Autoimmune alopecia
- Autoimmune chronic active hepatitis
- 35 Autoimmune hemolytic anemia
- Autoimmune neutropenia
- Autoimmune thrombocytopenic purpura

- Behçet's disease
- Cerebellar degeneration
- Chronic active hepatitis
- Chronic inflammatory demyelinating polyradiculoneuropathy
- 5 Chronic neuropathy with monoclonal gammopathy
- Classic polyarteritis nodosa
- Congenital adrenal hyperplasia
- Cryopathies
- Dermatitis herpetiformis
- 10 Diabetes
- Eaton-Lambert myasthenic syndrome
- Encephalomyelitis
- Epidermolysis bullosa acquisita
- Erythema nodosa
- 15 Gluten-sensitive enteropathy
- Goodpasture's syndrome
- Guillain-Barre syndrome
- Hashimoto's thyroiditis
- Hyperthyroidism
- 20 Idiopathic hemochromatosis
- Idiopathic membranous glomerulonephritis
- Isolated vasculitis of the central nervous system
- Kawasaki's disease
- Minimal change renal disease
- 25 Miscellaneous vasculitides
- Mixed connective tissue disease
- Multifocal motor neuropathy with conduction block
- Multiple sclerosis
- Myasthenia gravis
- 30 Opsoclonus-mycoclonus syndrome
- Pemphigoid
- Pemphigus
- pernicious anemia
- Polymyositis/dermatomyositis
- 35 Post-infective arthritides
- Primary biliary sclerosis
- Psoriasis

Reactive arthritides

Reiter's disease

Retinopathy

Rheumatoid arthritis

5 Sclerosing cholangitis

Sjögren's syndrome

Stiff-man syndrome

Subacute thyroiditis

Systemic lupus erythematosus

10 Systemic necrotizing vasculitides

Systemic sclerosis (scleroderma)

Takayasu's arteritis

Temporal arteritis

Thromboangiitis obliterans

15 Type I and type II autoimmune polyglandular syndrome

Ulcerative colitis

Uveitis

Wegener's granulomatosis

As used herein, the term "ester" refers to a group of the form -COOR , wherein R is a radical notionally derived from the alcohol ROH. Examples of ester groups include the physiologically hydrolysable esters such as the methyl, ethyl, n- and iso-propyl, n-, sec- and tert-butyl, and benzyl esters.

25 As used herein the term "alkylene" refers to a straight or branched alkyl chain having two unsatisfied valencies, for example $-\text{CH}_2-$, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, $-\text{CH}(\text{CH}_3)\text{CH}_2-$, $-\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_3$, and $-\text{C}(\text{CH}_3)_3$.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with up to four substituents, each of which independently may be (C₁-C₆)alkyl, trifluoromethyl, (C₁-C₆)alkoxy (including the special case where a ring is substituted on adjacent ring C atoms by methylenedioxy or ethylenedioxy), trifluoromethoxy, (C₁-C₆)alkylthio, phenyl, benzyl, phenoxy, hydroxy, mercapto, amino, fluoro, chloro, bromo, cyano, nitro, oxo, -COOH,

-SO₂OH, -CONH₂, -SO₂NH₂, -COR^A, -COOR^A, -SO₂OR^A, -NHCOR^A,
-NH₂SO₂R^A, -CONHR^A, -SO₂NHR^A, -NHR^A, -NR^AR^B, -CONR^AR^B or
-SO₂NR^AR^B wherein R^A and R^B are independently a (C₁-C₆)-
alkyl group, a (C₃ - C₇) cycloalkyl group or C₂ - C₆
5 alkoxy group. In the case where "substituted" means sub-
stituted by benzyl or phenoxy, the phenyl ring thereof
may itself be substituted with any of the foregoing,
except phenyl or benzyl.

As used herein the unqualified term "carbocyclyl" or
10 "carbocyclic" refers to a 5-8 membered ring whose ring
atoms are all carbon.

Some compounds of the invention contain one or more
chiral centres because of the presence of asymmetric
carbon atoms. The presence of asymmetric carbon atoms
15 gives rise to stereoisomers or diastereoisomers with R or
S stereochemistry at each chiral centre. The invention
includes all such stereoisomers and diastereoisomers and
mixtures thereof.

Salts of salt forming compounds of the invention
20 include physiologically acceptable acid addition salts
for example hydrochlorides, hydrobromides, sulphates,
methane sulphonates, p-toluenesulphonates, phosphates,
acetates, citrates, succinates, lactates, tartrates, fu-
marates and maleates; and base addition salts, for exam-
25 ple sodium, potassium, magnesium, and calcium salts.

In the compounds of the invention the following are
examples of the several structural variables:

Z may be, for example a carboxylic acid group
(-COOH) or a methyl or benzyl ester thereof. Presently
30 -COOH is preferred.

R₁ may be, for example, H, F, Cl, methyl, methoxy,
or methylenedioxy. Currently it is preferred that R₁ is
H, F, or Cl;

R₂ may be, for example cyclopropyl, phenyl, or
35 fluoro-, chloro-, methyl, methoxy-, nitro-, or amino-
substituted phenyl;

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their pharmacokinetic properties. The orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be

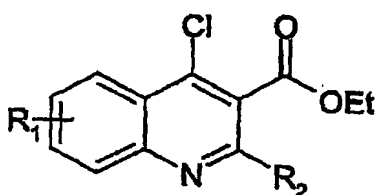
coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented
5 as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying
10 agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-
15 hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional
20 formulations well known in the art, for example as described in standard textbooks of pharmaceuticals such as the British Pharmacopoeia.

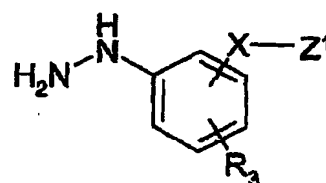
For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance
25 buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening
30 agents such as hypromellose may also be included.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants
35 such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

Compounds of the invention may be prepared by synthetic methods known in the literature, from compounds which are commercially available or are accessible from commercially available compounds. For example, compounds of formula (I) wherein Y is N may be prepared by reaction of a compound of formula (II) with an hydrazide of formula (III):



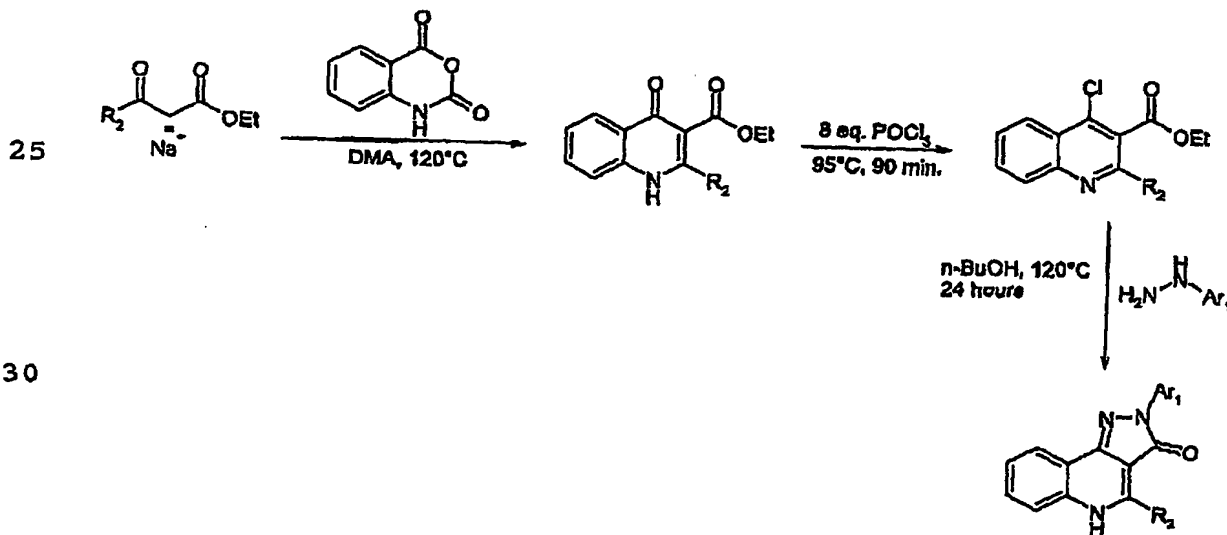
(II)



(III)

wherein Z1 is a carboxylic acid or an esterified carboxylic acid. Ester compounds (I) may of course be hydrolysed to the free acid.

The following Examples illustrate the preparation of compounds of the invention:
Synthetic route followed:

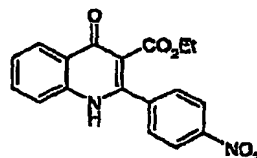


Typical experimental R_2 = 4-nitro phenyl, Ar_1 = 4-benzoic acid methyl ester

Step 1

2-(4-Nitro-phenyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester

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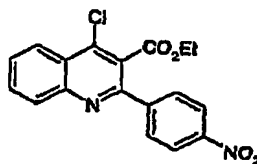


10 Sodium hydride (0.92 g, 0.023 mol; 60% suspension in
mineral oil) was added portionwise to a stirred solution
of 3-(4-nitrophenyl)-3-oxopropionic acid ethyl ester
15 (5.46 g, 0.023 mol) in dimethylacetamide (20 mL) at room
temperature. A solution of isatoic anhydride (3.4 g, 0.02
mol) in dimethylacetamide (20 mL) was added to this solu-
tion. The reddish mixture was stirred at 120 °C for 30
min and then the solvent was concentrated in vacuo. The
crude solid was partitioned between water and ethyl ace-
tate and the organic phase then separated. The combined
20 organic extracts were dried over sodium sulfate and con-
centrated in vacuo to leave a residue which was washed
once with cold tert-butylmethyl ether to yield 2-(4-
nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
ethyl ester (1.61 g, 28%) as a white solid, LCMS m/z
25 339.33 [M+H]⁺ @ R_T 1.16 min, 100% purity.

Step 2

4-Chloro-2-(4-nitro-phenyl)-quinoline-3-carboxylic acid
ethyl ester

30

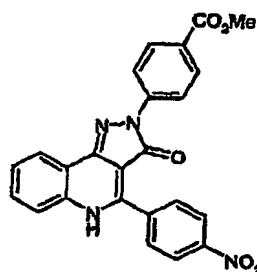


35 Phosphorus oxychloride (8 mL, 0.087 mol) was added in one portion to 2-(4-nitrophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (3.7 g, 0.0109 mol) and the mixture was heated at 95°C for 90 min. The

resulting light brown solution was added dropwise to a vigorously stirred ice-cold solution of sodium hydroxide (500 mL; 0.7 M). The aqueous suspension was extracted with ethyl acetate and the combined organic extracts were dried and concentrated in vacuo to leave 4-chloro-2-(4-nitrophenyl)-quinoline-3-carboxylic acid ethyl ester (3.8 g, 98 %) as a white solid, LCMS m/z 357.21 [M+H]⁺ @ R_T 1.94 min, 98% purity.

Step 3

4-[4-(4-Nitro-phenyl)-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl]-benzoic acid methyl ester

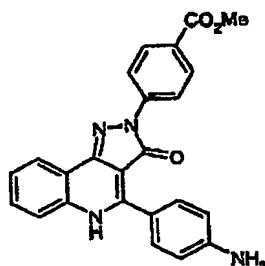


4-Chloro-2-(4-nitrophenyl)-quinoline-3-carboxylic acid ethyl ester (2.86 g, 0.008 mol) and 4-hydrazino-benzoic acid methyl ester hydrochloride (1.7 g, 0.008 mol) were stirred in *n*-butanol (70 mL) at 120 °C for 24 h. The bright orange suspension was diluted with tert-butylmethyl ether, filtered, washed with cold heptane and left to dry under suction to yield 4-[4-(4-nitrophenyl)-3-oxo-3,5-dihydropyrazolo[4,3-c]quinolin-2-yl]-benzoic acid methyl ester (2.7 g, 76 %) as an orange solid, LCMS m/z 441.35 [M+H]⁺ @ R_T 1.66 min: 84% purity.

Example 2

4-[4-(4-Amino-phenyl)-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl]-benzoic acid methyl ester

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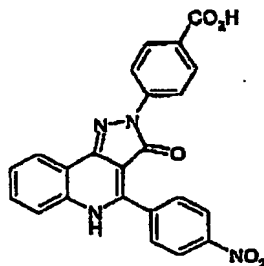


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4-[4-(4-Nitro-phenyl)-3-oxo-3,5-dihydro-pyrazo-
lo[4,3-c]quinolin-2-yl]-benzoic acid methyl ester (2.6 g,
10 5.9 mmol) and Pd/C (100 mg, 10%) were suspended in etha-
nol (150 mL) and acetic acid (6 mL) and stirred under
hydrogen for 24 h. The resulting yellow-orange suspension
was diluted with DMF (50 mL) and filtered. The solvent
was removed in vacuo to leave a residue which was washed
15 with methanol to give 4-[4-(4-amino-phenyl)-3-oxo-3,5-
dihydro-pyrazolo[4,3-c]quinolin-2-yl]-benzoic acid methyl
ester (2.0 g, 82 %) as a pale orange solid, LCMS m/z
411.39 [M+H]⁺ @ R_T 1.27 min, 79% purity.

Example 3

20 4-[4-(4-Nitro-phenyl)-3-oxo-3,5-dihydro-pyrazolo[4,3-
c]quinolin-2-yl]-benzoic acid



25

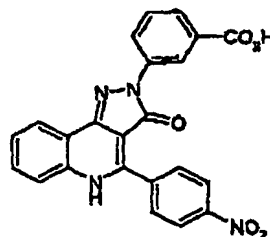
Prepared using the procedure described above, using
30 4-hydrazinobenzoic acid. LCMS m/z 427.34 [M+H]⁺ @ R_T 1.38
min, 74% purity

Example 4

3-[4-(4-Nitro-phenyl)-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl]-benzoic acid

35

15



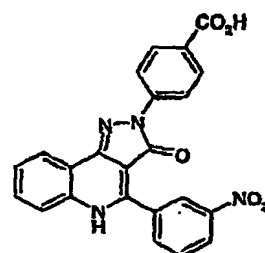
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Prepared by methods analogous to Example 3. LCMS m/z 427.37 [M+H]⁺ @ R_T 1.28 min, 96% purity.

Example 5

4-[4-(3-Nitro-phenyl)-3-oxo-3,5-dihydro-pyrazolo[4,3-c]-quinolin-2-yl]-benzoic acid

15

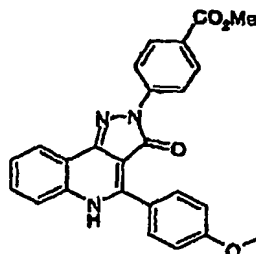


Prepared by methods analogous to Example 3. LCMS m/z 427.38 [M+H]⁺ @ R_T 1.33 min, 88% purity. δ_H (400 MHz, (CD₃)₂SO) 12.8 (1 H, s), 8.85 (1 H, t J 2.0), 8.54 (1 H, dd J₁ 7.1 J₂ 2.0), 8.35 (4 H, m), 8.02 (1 H, s), 8.0 (1 H, s), 7.94 (1 H, t J 8.0), 7.84 (1 H, d J 7.9), 7.74 (1 H, t, J 7.1), 7.6 (1 H, t J 7.1).

Example 6

4-[4-(4-Methoxyphenyl)-3-oxo-3,5-dihydropyrazolo[4,3-c]quinolin-2-yl]benzoic acid methyl ester

30



35

Prepared by methods analogous to Example 1. LCMS m/z 426.34 [M+H]⁺ @ R_T 1.71 min, 82% purity. δ_H (400 MHz, (CD₃)₂SO) 8.2 (2 H, d J 9.0), 8.05 (1 H, dd J₁ 8.0 J₂

fluorescent label 1, linker antibody 1, CD28 fusion protein, CD80 fusion protein, linker antibody 2, and fluorescent label 2. The table below describes these reagents in greater detail.

5

Fluorescent label 1	Anti-Rabbit IgG labelled with Europium (1 μ g/ml)
Linker antibody 1	Rabbit IgG specific for mouse Fc fragment (3 μ g/ml)
CD28 fusion protein	CD28 - mouse Fc fragment fusion protein (0.48 μ g/ml)
CD80 fusion protein	CD80 mouse Fab fragment (C215) fusion protein (1.9 μ g/ml)
Linker antibody 2	GoMk-biotin: biotinylated goat IgG specific for mouse kappa chain (2 μ g/ml)
Fluorescent label 2	SA-APC: streptavidin labelled allophycocyanin (8 μ g/ml)

On formation of the complex, europium and APC are brought into proximity and a signal is generated.

10 Non-specific interaction was measured by substituting a mouse Fab fragment (C215) for the CD80 mouse Fab fragment fusion protein (1.9 μ g/ml). The assay was carried out in black 384 well plates in a final volume of 30 μ l. Assay buffer: 50mM Tris-HCl, 150mM NaCl pH7.8, containing 0.1% BSA (w/v) added just prior to use.

15 Compounds were added to the above reagents in a concentration series ranging between 100 μ M - 1.7nM. The reaction was incubated for 4 hours at room temperature. Dual measurements were made using a Wallac Victor 1420 Multilabel Counter. First measurement: excitation 340nm, 20 emission 665nm, delay 50 μ s, window time 200 μ s. second measurement: excitation 340nm, emission 615nm, delay 50 μ s, window time 200 μ s. Counts were automatically corrected for fluorescence crossover, quenching and background.

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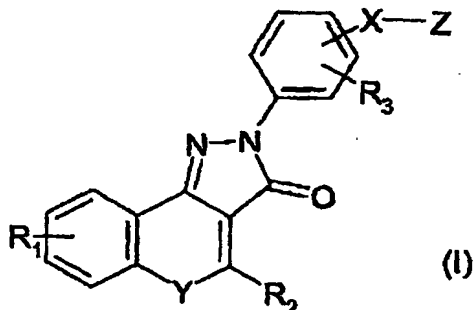
By way of illustration, the IC_{50} results for the compounds of Examples 5, 7 and 9 were 8.6 μM , 3.4 μM and 4.6 μM respectively.

CLAIMS

1. A compound of formula (I) or a pharmaceutically or veterinarily acceptable salt thereof:

5

10



wherein

Z represents a carboxylic acid group (-COOH) or an ester thereof;

15

R1 and R3 independently represent H; F; Cl; Br; -NO2; -CN; C1-C6 alkyl optionally substituted by F or Cl; or C1-C6 alkoxy optionally substituted by F;

R2 represents optionally substituted C3-C7 cycloalkyl or optionally substituted phenyl;

20

Y represents -O-, -S-, N-oxide, or -N(R5)- wherein R5 represents H or C1-C6 alkyl;

X represents a bond or a group selected from; a divalent C1-C6 alkylene radical, NHC(O) C1-5 alkyl or NHC(O) CH2-O-CH2

25

2. A compound as claimed in claim 1 wherein X is a bond or a -CH2- or -CH2CH2- radical.

3. A compound as claimed in claim 1 or claim 2 wherein Z is -COOH.

30

4. A compound as claimed in any of the preceding claims wherein R1 is H, F, Cl, methyl, methoxy, or methylenedioxy.

5. A compound as claimed in any of the preceding claims wherein R2 is cyclopropyl, phenyl, or fluoro-, chloro-, methyl, methoxy-, nitro-, or amino- substituted phenyl

35

6. A compound as claimed in any of the preceding claims wherein R_3 is H, F, Cl, methyl, methoxy, or methylenedioxy.

5 7. A compound as claimed in any of the preceding claims wherein Y is $-N(R_5)-$ wherein R_5 represents H or methyl.

8. A compound as claimed in any of claims 1 to 7 for use in the treatment of conditions which benefit from immunomodulation.

10 9. The use of a compound as claimed in any of claims 1 to 7 in the manufacture of a medicament for the treatment of conditions which benefit from immunomodulation.

15 10. A method of immunomodulation in mammals, including humans, comprising administration to a mammal in need of such treatment an immunomodulatory effective dose of a compound as claimed in any of claims 1 to 9.

20 11. A pharmaceutical or veterinary composition comprising a compound as claimed in any of claims 1 to 9 together with a pharmaceutically or veterinarily acceptable excipient or carrier.

12/16/02

